-40, -78, -136, and -196 °C), a yellow liquid **11** was found at -78 °C (~68% yield). The residue in the flask was dissolved in dry acetone (20 mL). The solution was filtered and the filtrate reduced to dryness, leaving a solid compound **22** (~22% yield).[24] The spectral data obtained for **11** are as follows: ^{19}F NMR ϕ -96.0; ^{1}H NMR, complex—no N-H resonance present; MS (EI, 10 eV) [m/e (species) intensity] 159 (M^+) 2.04, 144 ($M^+ - CH_3$) 100, 140 (M^+ - F) 94, 139 (M^+ - HF) 1.1, 109 ($C_7H_{11}N^+$) 31.57, 76 ($C_3H_7NF^+$) 20.12, 56 ($C_3H_6N^+$) 27.8; IR (liquid film) 2972 vs, 2942 vs, 2872 s, 1465 vs, 1449 vs, 1380 vs, 1368 s, 1125 s, 1033 m, 899 m, 735 w, 686 w, br, 669 w, and 540 w, cm^{-1}.

(l) 2,2,6,6-$(CH_3)_4$-c-C_5H_6NF (11) + PF_5 To Form [$\overline{CH_2CH_2CH_2}$-$\overline{C(CH_3)_2N^+}$=$C(CH_3)_2$]PF_6^- (23). Methylene chloride (10 mL) and 0.329 g (2 mmol) of 2,2,6,6-$(CH_3)_4$-c-C_5H_6NF (**11**) were combined in a dry 100-mL round-bottomed flask equipped with a Kontes Teflon stopcock and a Teflon-coated stirring bar. The vessel was evacuated at -196 °C, and 0.174 g (2 mmol) of phosphorus pentafluoride was condensed into the flask. The mixture was warmed to room temperature and stirred for 12 h. The solvent was evaporated under vacuum, and the resulting white solid **23** (~90% yield) was recrystallized from a mixture of acetonitrile/hexane/ether (2:1:1). The spectral data obtained for **23** are as follows: ^{19}F NMR ϕ -72.82 d (J_{P-F} = 708 Hz); ^{31}P NMR δ -145.04 sept; ^{1}H NMR δ 2.4 m (6 H), 1.5 m (6 H), 1.7 m (6 H); MS (FAB$^+$, glycerol) [m/e (species) intensity] 140 ($M^+ - PF_6$) 100; MS (FAB$^-$, glycerol) [m/e (species) intensity] 145 (PF_6^-) 100; IR (KBr pellet) 2940 vs, 2870 s, 1660 w, 1590 s, 1470 s, br, 1380 m, 1360 w, 1320 m, 1270 m, 1230 m, 1130 m, 930 m, 920 m, 720 w, 510 w, 470 m, cm^{-1}.

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Registry No. 1, 14722-43-1; **2**, 125227-79-4; **3**, 125227-80-7; **4**, 125227-81-8; **5**, 125227-82-9; **6**, 125227-83-0; **7**, 125227-84-1; **8**, 125227-85-2; **9**, 17265-86-0; **10**, 24151-83-5; **11**, 24192-33-4; **12**, 62-75-9; **13**, 55-18-5; **14**, 621-64-7; **15**, 601-77-4; **16**, 924-16-3; **17**, 997-95-5; **18**, 947-92-2; **19**, 930-55-2; **20**, 100-75-4; **21**, 17721-95-8; **22**, 6130-93-4; **23**, 125227-87-4; NF_3O, 13847-65-9; Me_2NH, 124-40-3; Et_2NH, 109-89-7; Pr_2NH, 142-84-7; i-Pr_2NH, 108-18-9; Bu_2NH, 111-92-2; i-Bu_2NH, 110-96-3; (c-$C_6H_{11})_2NH$, 101-83-7; c-C_4H_8NH, 123-75-1; c-$C_5H_{10}NH$, 110-89-4; 2,6-$(CH_3)_2$-c-C_5H_8NH, 504-03-0; 2,2,6,6-$(CH_3)_4$-c-C_5H_6NH, 768-66-1; PF_5, 7647-19-0.

Gilvocarcin Photobiology.[1] Isolation and Characterization of the DNA Photoadduct

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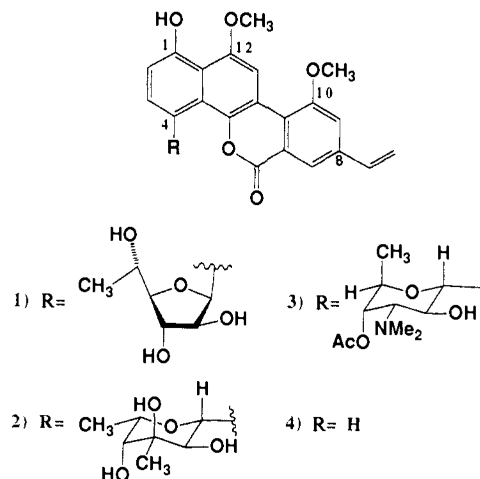
Abstract: Gilvocarcin V forms a covalent adduct with double-stranded DNA under the influence of light. The structure of this covalent modification is identified as a [2 + 2] cycloadduct between the vinyl group of gilvocarcin V and a thymine residue. The stereochemistry of this adduct is deduced from NMR.

Gilvocarcin V (**1**),[2,3] also known as toromycin[4] and anandimycin,[5] is representative of a new class of aromatic C-glycoside antibiotics possessing significant antitumor activity.[6] Other members of this family include chrysomycin A (**2**)[7] (albacarcin V,[8] virenomycin V[9]) and ravidomycin (**3**),[10] which incorporate the same aromatic aglycon attached to various glycosidic substituents. The aglycon of these antitumor antibiotics, defucogilvocarcin V[11] (**4**), has recently been isolated as a natural product. This aglycon has been the subject of a number of synthetic studies[12-16] including our own.[17] The related natural product, gilvocarcin M (**5**), possesses an aglycon in which the vinyl substituent at C8 is replaced by a methyl group.



Although gilvocarcin V is known to inhibit DNA synthesis,[18] little is known about the precise mechanism of action. The extreme potencies of some of the gilvocarcins in cell culture and in vitro tests, where DNA strand scission has been observed,[19] stand in sharp contrast to the apparent tolerance of whole animals for large doses. Elespuru and Gonda[20] identified light exposure of the gilvocarcins in the presence of DNA as a significant requirement for potency in cell culture and in vitro tests such as DNA strand nicking. Gilvocarcin V is 10^3–10^5 times more active than the photoactive psoralens, 8-methoxypsoralen (**6**) and trioxsalen (**7**), in the prophage-induction assay for DNA damage.[20] The wavelength dependence of the λ prophage induction assay with gilvocarcin V correlates strongly with the absorption spectra of the natural product.[21] Similar light-dependent activity has been observed with the ravidomycin antibiotics.[22] Under visible light, gilvocarcin V is capable of generating both singlet oxygen and

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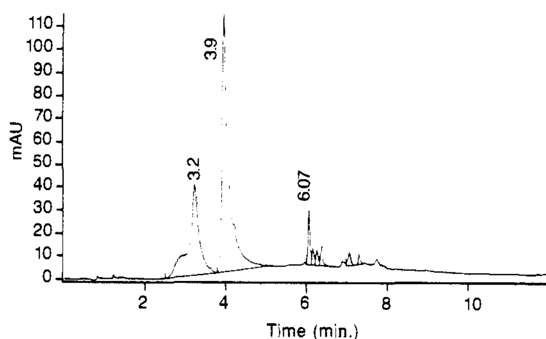
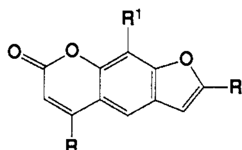


Figure 1. HPLC of DNA digest from Gilvocarcin V–DNA photolysis experiment, 260 nm detection, HP-ODS HPLC column, 100 mm × 3.4 mm, 0–100% methanol in water at 10%/min, flow 1 mL/min. The photoadduct **8** corresponds to the peak at 6.07 min. Control experiments are illustrated in the supplementary material.

hydroxyl radicals that could contribute to the phototoxicity.[23] Biological studies in this series are complicated by experiments in which light activation was not taken into consideration. One report,[24] for example, describes strong binding to DNA by gilvocarcin V without DNA nicking but no mention is made of light or any efforts to exclude light.



6) R=H-, R^1=CH_3O-
7) R=CH_3-, R^1=CH_3-

Our preliminary studies[25] on the mechanism of action of the gilvocarcins further defined the role of light activation. We found that gilvocarcin V intercalates into DNA in the dark and that strand nicking and covalent modification occur only in the presence of light. Formation of a covalent adduct between gilvocarcin V and DNA was initially detected by a change in the electrophoretic mobility of plasmid DNA and short thymidine-containing double-stranded oligonucleotides. These experiments demonstrated a requirement for double-stranded DNA. The glycosyl group of gilvocarcin V is also required for efficient binding to be observed. Additional studies[26] with radiolabeled gilvocarcin V are consistent with these results. In vivo studies in human cells suggest that gilvocarcin V may photo-cross-link DNA and protein.[27]

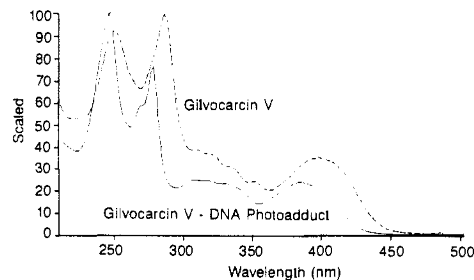


Figure 2. UV spectra of gilvocarcin V (dotted line) and the DNA photoadduct **8** (solid line).

In analogy to the photoactive psoralens,[28] a model of the intercalation complex[29] of gilvocarcin V in double stranded DNA suggests that a [2 + 2] photocycloadduct may form between gilvocarcin V and a pyrimidine nucleotide.[30] To provide a better understanding of the mechanism of action of the gilvocarcins, we undertook the isolation and characterization of this covalent adduct.

Results and Discussion

A solution of gilvocarcin V in DMSO was added to a buffered solution of calf-thymus DNA, and the solution was photolyzed with low-energy (>300 nm) UV light. After precipitation with ethanol, the DNA treated with gilvocarcin V and light had a yellow color. In contrast, the DNA photolyzed in the absence of gilvocarcin V was white and DNA precipitated in the presence of gilvocarcin V in the absence of light was a very faint yellow.

The resulting DNA pellets were digested with 0.1 N HCl at 100 °C for 2 h. These conditions are sufficient to hydrolyze the glycosidic bond of purine residues as well as pyrimidine residues in which the 5,6-double bond is missing.[31] When samples of these hydrolysis reactions were compared by HPLC, four new peaks (Figure 1) were observed that were not present in the control experiments. These new compounds are less polar than the control DNA fragments and more polar than gilvocarcin V. UV spectra (Figure 2), recorded during the chromatography, showed the presence of a chromophore similar to that of gilvocarcin M.[3] This blue shift suggests that the vinyl group of gilvocarcin V is no longer in conjugation with the aromatic ring system.

The four new compounds could represent stereoisomers of the proposed cyclobutane ring, although modeling of the intercalation site suggests only one stereoisomer will form. Alternatively, they could be the result of acid-catalyzed isomerization of the fucosyl group to an equilibrium mixture of the four possible furanose and pyranose stereoisomers. Such acid-mediated isomerization has been demonstrated for gilvocarcin V (eq 1). Three of the four possible glycosyl isomers of gilvocarcin V have been identified.[32] Under the conditions of DNA digestion similar isomerization should be expected for the DNA photoadduct.

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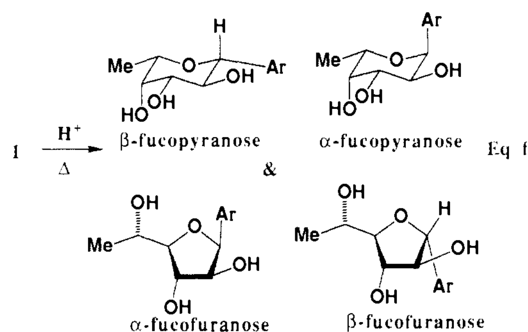
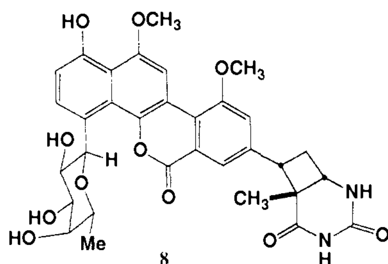


The compounds responsible for the new peaks were first isolated from the DNA fragments as a single fraction by filtration through a C18 cartridge. HPLC analysis of this fraction gave the ratio of the four photoadducts as 6:73:11:11. The major component was isolated by preparative reverse-phase liquid chromatography and fully characterized. This compound was assigned structure **8** as described below. The pyranose ring in **8** confirms that isomerization has occurred. Under conditions of partial DNA hydrolysis (20 mn), a nonequilibrium ratio of the four isomers is obtained in which the native β-furanose configuration predominates. Resubmittal of this sample to the conditions of DNA digestion results in the equilibrium ratio of isomers.



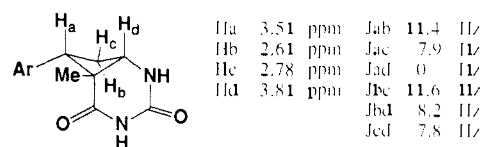
FAB mass spectral analysis of **8** shows in the high-mass region ions at m/z 643 $[M + Na]^+$ and 620 $[M]^+$ assignable to a gilvocarcin V–thymine adduct plus Na^+ and a gilvocarcin V–thymine adduct, respectively. Major fragment ions observed at m/z 517 and 487 correlate with dehydration followed by retro-hetero-Diels–Alder fragmentation of the pyranose ring.

In the NMR spectrum of compound **8**, the aromatic nucleus of the gilvocarcin system is readily discerned, but no vinyl proton signals are present. Instead, there is a coupled system of four multiplets in the aliphatic region that are assigned to the cyclobutane subunit. In addition, the sharp singlet for the thymidyl methyl group at 1.64 ppm compares well with psoralen photoadducts of thymine where the corresponding signal appears in the range of 1.50–1.76 ppm.[28] The signal for the anomeric H(1′) proton is clearly visible at 5.85 ppm as a doublet with a coupling of 9.3 Hz, consistent with the α-pyranose configuration[32] of the glycosyl residue.

In the NMR spectrum of the mixture of isomers, small spectral shifts are apparent at the signal for the C3 proton of the aromatic A ring, consistent with isomerization at the glycosyl center. The D-ring proton signals are unshifted, indicating a common cyclobutyl ring stereochemistry.

There are four possible stereoisomers of the cyclobutane ring. These are syn and anti with respect to the aryl and thymidyl rings and 1,3- vs 1,4-attachment of the aryl group on the cyclobutane ring. Only one of these is consistent with the intercalation model. Each possible isomer has, in addition, two potential conformational extremes which correspond to axial and equatorial orientations of the aryl group on the cyclobutyl ring.

The question of 1,3- vs 1,4-attachment of the aryl group to the cyclobutane can be approached via NMR coupling constant data. This analysis is complicated by the need to consider possible large (5–9 Hz) transannular couplings which occur in many cyclobutane derivatives.[33-35] The benzylic proton, Ha, and the proton on the carbon bearing the nitrogen atom, Hd, exhibit no coupling to each other. Each is coupled with large coupling constants to the methylene protons, Hb and Hc, which share a large geminal coupling constant. Dihedral angles between vicinal protons were measured from Dreiding models[36] for each of the eight possible conformations. Application of the Karplus equation as modified by Bothner-By[37] reveals that all configurations except one have at least one dihedral angle which predicts a small (2–4 Hz) coupling constant. Only for the syn 1,3 isomer, illustrated in Figure 3, in the conformation appropriate for the lack of long range coupling are all vicinal coupling constants expected to be large (8–12 Hz). This conformation places the two protons Ha and Hd in axial positions, allowing the aryl group to adopt an equatorial orientation. Further corroboration is obtained from 2D NOE experiments.[38] A small but detectable NOE is observed between Ha and Hd. No NOE is observed between the methyl substituent and any of the cyclobutyl protons.



Ha	3.51 ppm	Jab	11.4 Hz
Hb	2.61 ppm	Jac	7.9 Hz
Hc	2.78 ppm	Jad	0 Hz
Hd	3.81 ppm	Jbc	11.6 Hz
		Jbd	8.2 Hz
		Jcd	7.8 Hz

Figure 3. Relative configuration and conformation of the cyclobutyl portion of **8**, with the corresponding ^1H NMR chemical shifts and coupling constants.

This is consistent with the syn 1,3 isomer in the conformation with Ha and Hd axially disposed across the ring from each other with the aryl group and methyl group equatorial as illustrated. The relative stereochemistry between the cyclobutane and the remote fucose ring was not established.

Conclusions

The in vitro generated covalent photoadduct between gilvocarcin V and DNA has been isolated and characterized as the product of a [2 + 2] photocycloaddition between the vinyl group of gilvocarcin V and a thymidyl residue on DNA. The stereochemistry of the cyclobutyl ring of this photoadduct has been elucidated. The syn 1,3 isomer is the only isomer consistent with the NMR data. This is the isomer expected from photoreaction of the intercalation complex of gilvocarcin V in DNA with the vinyl substituent in proximity to the double bond of a thymidine residue.

Experimental Section

The following abbreviations are used: DMSO, dimethyl sulfoxide; DNA, deoxyribonucleic acid; FAB, fast atom bombardment; HPLC, high-performance liquid chromatography; IR, infrared; MPLC, medium-pressure liquid chromatography; MS, mass spectrum; NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect; RP, reverse phase; UV, ultraviolet.

NMR spectra were measured on a GE QE300 or a Bruker WM400 NMR spectrometer. Chemical shifts are reported as δ values relative to internal tetramethylsilane or to the residual solvent signals in $CDCl_3$ at 7.26 ppm or in d_6-DMSO at 2.52 ppm. UV spectra were recorded during analytical HPLC on a Hewlett-Packard 1090m chromatograph with an HP1040 diode array detector. The FAB mass spectrum was taken on a VG ZAB-SE multifocusing high-resolution mass spectrometer.

Photoadduct Preparation and Isolation. Gilvocarcin V was prepared by fermentation and isolated according to the reported procedure.[2,3,11] This affords gilvocarcin V 90% pure by HPLC. The major contaminant is gilvocarcin M, coproduced in the fermentation. Two recrystallizations from dry hot acetone–methanol yield gilvocarcin V as a yellow crystalline powder which was 99% pure by HPLC, mp 244–247 °C dec.

Samples of calf-thymus DNA (Sigma) or herring sperm DNA (Fluka), 20 mg each, were dissolved in 20 mL of Tris buffer (10 mM

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TRIZMA base, 1 mM Na, EDTA, pH 8.0). Gilvocarcin V was dissolved in DMSO to a concentration of 1 mg/mL. In a darkened room, 1 mL of the gilvocarcin V solution was added to each sample of DNA solution. After vigorous shaking, the samples were placed in the center chamber of a double-jacketed photolysis apparatus. The outer chamber contained a precooled 40% solution of cobalt nitrate in water to act as a filter and as a temperature buffer. The samples were photolyzed for 30 min in a Rayonet photochemical reactor equipped with 16 General Electric F8T5-BLB 75-W lamps. After photolysis, 3 mL of saturated NaCl solution was added, and the mixture was poured into 70 mL of cold ethanol. The resulting precipitate was wound onto a glass rod and pressed to remove additional ethanol. Control experiments were conducted both with drug while omitting light exposure and with light while omitting drug. The precipitated DNA pellets were white for the control without drug and yellowish off-white for the control excluding light. The experimental pellets were a yellow-orange color. HPLC analysis of the ethanol supernatant revealed that, in comparison to the light excluded control, 75% of the added gilvocarcin V was bound to the DNA precipitate after light exposure and that the unbound gilvocarcin V was unchanged.

DNA adducts were isolated by hydrolyzing the combined DNA pellets from 16 photolysis experiments with 10 mL of 0.1 N HCl at 100 °C for 2 h. This solution was filtered through Waters C-18 Sep-Pak cartridges. The cartridges were rinsed with water and increasing percentages of methanol in water. Adducts containing the gilvocarcin M chromophore eluted with 60-70% methanol in water. In practice, after eluting with 40% methanol, the cartridges were washed with 100% methanol to elute the desired fraction in a small volume which was concentrated under vacuum. HPLC analysis revealed a mixture of four isomers in a ratio of 6:73:11:11. These were fractionated by semipreparative reverse-phase MPLC using an EM Science LoBar C-8 size A column, eluting with 50% methanol-water at 3 mL/min. The major component **8** was separated from the other isomers. The latter two isomers were characterized by NMR analysis of the mixture as the furanose isomers. The minor component was not obtained in sufficient quantity for further characterization.

Gilvocarcin V-DNA Photoadduct. β-Fucopyranose isomer **8**: HPLC (HP-ODS column 3.4 mm × 100 mm, 60% methanol in water, 1 mL/min, 260-nm detection) 1.7 min; ^{1}H NMR (d_4-methanol, 300 MHz) δ 8.61 (s, 1 H), 7.88 (d, 1 H, J = 8.4 Hz), 7.87 (br s, 1 H), 7.40 (br s, 1 H), 6.99 (d, 1 H, J = 8.7 Hz), 5.85 (d, 1 H, J = 9.3 Hz), 4.36 (q, 1 H, J = 6.0 Hz), 4.17 (s, 6 H), 4.14 (m, 1 H), 3.82 (m, 2 H), 3.54 (m, 2 H), 2.75 (m, 1 H), 2.59 (m, 1 H), 1.62 (s, 3 H), 1.23 (d, 3 H, J = 6.3 Hz); ^{1}H NMR (10% d_4-methanol in $CDCl_3$, 400 MHz) δ 8.59 (s, 1 H), 7.89 (br s, 1 H), 7.88 (d, 1 H, J = 8.5 Hz), 7.30 (d, 1 H, J = 1.7 Hz), 7.04 (d, 1 H, J = 8.4 Hz), 5.83 (d, 1 H, J = 9.6 Hz), 4.40 (q, 1 H, J = 6.6 Hz), 4.19 (t, 1 H, J = 10 Hz), 4.19 (s, 3 H), 4.17 (s, 3 H), 4.12 (br s, 2 H), 3.89 (s, 1 H), 3.87 (dd, 1 H, J = 10, 3.5 Hz), 3.81 (t, 1 H, J = 8.0 Hz), 3.51 (dd, 1 H, J = 11.5, 8.0 Hz), 2.78 (ddd, 1 H, J = 11.5, 8.0, 8.0 Hz), 2.61 (ddd, 1 H, J = 11.5, 11.5, 8.0 Hz), 1.68 (s, 3 H), 1.28 (d, 3 H, J = 6.6 Hz); FAB MS (argon bombardment in a matrix of glycerol-thioglycerol) m/z M^+ ($C_{32}H_{32}O_{11}N_2$) 620.09 (54), $[M + Na]^+$ 643.1 (10), 517.05 (26), 487.05 (100).

α-Fucofuranose isomer: HPLC 2.1 min; ^{1}H NMR (d_4-methanol, 300 MHz) δ 8.52 (s, 1 H), 7.85 (d, 1 H, J = 8.1 Hz), 7.84 (br s, 1H), 7.38 (br s, 1 H), 6.95 (d, 1 H, J = 8.4 Hz), 6.07 (d, 1 H, J = 0.9 Hz), 4.15 (s, 6 H), 3.82 (m, 1 H), 2.77 (m, 1 H), 2.59 (m, 1 H), 1.64 (s, 3 H), 1.32 (d, 3 H, J = 6.6 Hz).

β-Fucofuranose (natural) isomer: HPLC 2.6 min; ^{1}H NMR (d_4-methanol, 300 MHz) δ 8.51 (s, 1 H), 8.11 (d, 1 H, J = 8.4 Hz), 7.82 (br s, 1 H), 7.35 (br s, 1 H), 6.96 (d, 1 H, J = 8.7 Hz), 6.34 (d, 1 H, J = 3.9 Hz), 4.16 (s, 6 H), 3.82 (m, 1 H), 2.77 (m, 1 H), 2.59 (m, 1 H), 1.64 (s, 3 H), 1.41 (d, 3 H, J = 6.6 Hz).

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Supplementary Material Available: Figures of the HPLC traces of the DNA hydrolysis reactions and the UV, FAB MS, and NMR spectra of compound **8** (9 pages). Ordering information is given on any current masthead page.

Chiral Synthesis via Organoboranes. 24. *B*-Allylbis(2-isocaranyl)borane as a Superior Reagent for the Asymmetric Allylboration of Aldehydes

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Abstract: Hydroboration of (+)-2-carene, readily available via the base-induced isomerization of (+)-3-carene, provides bis(2-isocaranyl)borane, which can be readily transformed into *B*-allylbis(2-isocaranyl)borane (2-^{d}Icr$_2$BAll). This new reagent undergoes asymmetric allylboration with a variety of aldehydes and affords the corresponding homoallylic alcohols in 94-99% ee. The enantioselectivities realized with this reagent are significantly higher than those realized with the previously explored reagents, *B*-allyldiisopinocampheylborane (^{d}Ipc$_2$Ball) and *B*-allylbis(4-isocaranyl)borane (4-^{d}Icr$_2$BAll).

Over the past few years, asymmetric allyl- and crotylboron reagents have proven to be exceptionally valuable in the context of acyclic stereoselection. Driven by the rapidly growing demand for highly enantiomerically pure substances in multistep natural product syntheses,[1] the development of superior allylboron reagents, which can achieve enantio- and diastereoselectivities approaching 100%, has evidently become both desirable and challenging.[2] Consequently, in continuation of our efforts in this

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